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Influence of period light on cultivation of microalgae consortium for the treatment of tannery wastewaters from leather finishing stage

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ABSTRACT

Wastewater from the leather finishing step in tanneries contains high nutrient loads and toxic compounds, such as dyes, acids, toxic metals among others. To our best knowledge, for the first time, the influence of light period on the treatment of wet-end and finishing leather wastewaters with a microalgae consortium containing mainly *Tetraselmis* sp. was investigated. In this work, a microalgae consortium present the ability to grow well in this kind of tannery wastewater and was used for reducing nitrogen, phosphorus, ammonium, chemical and biochemical oxygen demands. Additionally, the wastewater presents high turbidity, which has an influence on the penetration of light, during the cultivations. Therefore, the influence of the illumination period was also studied: continuous light (24-light), 12 h light/dark (12-light) and without light (0-light); in addition to the influence of compositions, 50% and 75% of raw wastewater. The maximum concentration of biomass in the wastewater was observed in the 24-light culture: 50R50S (1.40 gL^{-1}) and 75R25S (1.04 gL^{-1}), with maximum removals of total nitrogen (71.74%), total phosphorus (97.64%), total organic carbon (31.35%), chemical oxygen demand (56.70%), biological oxygen demand (20.68%) and ammonium (100%). These results show that this microalgae consortium is a promising alternative treatment for wastewaters of the leather wet-end and finishing steps.

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1. Introduction

The leather processing industry generates enormous amounts of wastewater that are typically characterized by high organic load, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total organic carbon (TOC), dissolved organic carbon (DOC), high suspended solids, organic nitrogen and ammonia, washing water durability, high salinity (total dissolved levels) (Sawalha et al., 2019; Tamersit et al., 2018). Therefore, this effluent is highly toxic, posing a risk to human, animal and plant health if improperly disposed in the environment.

The pollution loads of tannery effluent vary widely and depend on the raw materials and chemicals used, as well as the leather processing steps. The processing steps of the transformation of hide into leather are divided into three main stages as it follows (Puchana-Rosero et al., 2018):

A. Beamhouse stage: salt shake-off to pickling steps. Chemical processes are carried out in drums with water addition. Mechanical operations are also performed;

B. Tanning stage: carried out in drum with water addition and tanning agent;

C. Finishing stage: wet-end processing in drums, followed by drying, pre-finishing and final finishing treatments on the leather surface.

The finishing stage is carried out to give the leather the esthetic characteristics such as soft, color, and shine. Large amounts of water and a variety of chemicals in the wet-end steps performed in drums or by the application of coating products on the leather surface during the finishing (Tamilselvi et al., 2019). Table 1 brings a description and the chemicals added in wet-end and finishing. The

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Table 1
Wet-end and leather finishing steps.

Process	Steps	Purpose	Chemicals (added in water medium)	References
Wet-end	Acid washing and deacidification	Mild acid washing to remove remains of chrome from the surface (chrome that was not completely linked to the collagen fibers during the tanning). The deacidification neutralizes acids present in wet-blue leather.	• oxalic acid • neutralizing tannins • sodium bicarbonate • sodium formate	(Gomes et al., 2016; Sathish et al., 2019)
	Retanning	Treatment to obtain physical-mechanical, texture characteristics and desired properties of the leather through the use of retanning substances	• chrome, aluminum and zirconium salts • vegetable tannins • synthetic tanning materials (syntans), polymers (resins) and other retanning possibilities	(Benvenuti et al., 2018; Duan et al., 2019)
	Dyeing	To impart sensory characteristics of the final product, such as color shade and intensity, color penetration inside the leather, color uniformity, chemical and physical fastness	• dyestuffs (mainly acid and azo dyes); • dyeing auxiliaries that are leveling; • penetrating agents	(Fuck et al., 2018; Ortiz-Monsalve et al., 2019)
	Fatliquoring	The leather fibers are lubricated with oils to give softness to the leather and to prevent the fiber structure resticking during drying	• Sulfated anionic fatliquors • sulfited anionic oils • synthetic fatliquors • self-emulsifying synthetic raw oil • lecithins • phosphated fatliquors • surface active agents (emulsifiers) • formic acid	(Onem, 2018; Sun et al., 2018)
Finishing	Leather coating	Surface treatments to figure to the leather the visual and definitive aspects: application of covering, form-film products and top layers	• Polymers: mainly polyurethanes and polyacrilates • Other polymers like casein, vinyl, polybutadiene, nitrocellulose, acetobutyrate, and hybrid resins • Pigments, crosslinking agents solvents, diluents, auxiliary products (penetrators, thickeners, matting, hyrophobic, and touch agents)	(Han et al., 2019; Lai et al., 2017; Winter et al., 2018)

composition of the wastewater generated in type C tanneries is variable and depends on many factors related to the characteristics and quality requirements of the final finished leather for the trade market and its specifications (Gutterres et al., 2015; Mella et al., 2019). The wastewater from the leather industry is usually subjected to mechanical (Romero-Dondiz et al., 2016), physical-chemical (Kuppusamy et al., 2017) and biological treatments (Ortiz-Monsalve et al., 2019). However, wastewaters from the finishing stage are difficult to treat by conventional methods because of the presence of recalcitrant chemicals, such as syntans, resins, fatliquors, and dyes.

Although most of these chemicals are bonded in the leather, a portion is discarded with the wastewater. Studies show that only 80% of the dyes (Zanoni and Yanamaka, 2016) and 60–70% chrome (Sawalha et al., 2019) are fixed in the hides. The wastewaters generated in the tanning step can be recycled (Aquim et al., 2019). However, other wastewater streams must be adequately treated to be discharged in water bodies without eutrophication risks and water pollution.

Microalgaes were presented in several studies of wastewater treatment as a viable alternative for the depletion of various nutrients (Ran et al., 2019), organic matter (Song et al., 2020) and heavy metals (Urrutia et al., 2019). The microalgae cultivation has recently been applied in the treatment of municipal (Aketo et al., 2020), industrial (Nayak and Ghosh, 2019), agricultural (Khalid et al., 2019) and livestock wastewater (Li et al., 2020). However, tannery wastewater treatment conducted with microalgae is still limited. The high chemical load, turbidity and salinity in the raw tannery wastewater restricts the growth of microalgae (Saranya and Shanthakumar, 2019). Therefore, some applications of microalgae in tannery wastewater were carried out with pre-treated wastewater (Saranya and Shanthakumar, 2020), diluted (Fontoura et al., 2017) or added culture medium (Sundaramoorthy et al., 2016).

Some researchers used the wastewater generated in complete leather processing. Ajayan et al. (2015) studied the cultivation of microalgae *Scenedesmus* sp. for 12 days in tannery wastewater (10%, 25%, 50%, 75%, and 100%) with continuous illumination 4000 lux. The removal of chromium, copper, lead, and zinc was investigated. Maximum cell growth of 1180×10^4 cells/mL was observed in 50% tannery wastewater at 8th day with the removal of toxic metals above 70%. Another study using microalgae *Scenedesmus* sp. and three different dilutions of wastewater from complete leather manufacturing (20%, 50%, and 100%) assess the removal of hexavalent chromium, nutrients (nitrites, nitrates, phosphates and sulfates), and BOD. The microalgae growth was proportional to effluent concentration with higher growth in the effluent without dilution and removal of chrome (>98%), nitrates (>90%) and phosphates (>99%) and BOD (>88%) (Ballen-Segura et al., 2016). Fontoura et al. (2017) also applied the microalgae *Scenedesmus* sp. in the treatment of raw wastewater from the beamhouse stage of a the leather industry. Different concentrations of beamhouse wastewater (between 20% and 100%) were used with different light intensities (between 80 and 200 μmol of photons $m^{-2}\,s^{-1}$). The best removal results were obtained with 88.4% effluent, reaching the maximum biomass concentration 0.90 g L^{-1}, maximum ammonia nitrogen removal 85.63%, phosphorus 96.78% and COD 80.33%.

Others microalgaes have also been used to treat tannery wastewater. *Arthrospira* was used in effluent generated in the beamhouse stage to wet-blue (Dunn et al., 2013), *Chlorella vulgaris* (Das et al., 2017) and microalgae consortium containing *Chlorella* sp. and *Phormidium* sp. (Das et al., 2018) were used in the wastewater of complete process.

Minimal researches are found in the literature regarding the

treatment of wastewater from the leather finishing stage using microalgae. In the authors previous study, wastewater from the finishing stage (raw wastewater, after primary and secondary treatment) was treated with a microalgae consortium. In this study it was noticed that there were removals close to P-PO_4, NTK (Total Kjeldahl Nitrogen), N−NH_3, and COD among the compared wastewater and a higher growth of microalgae in the raw wastewater (Pena et al., 2019). In another study from authors, Pena et al. (2018) used the microalgae *Tetraselmis* sp. isolated from a microalgae consortium. It evaluated for their ability for the wastewater treatment collected from the tannery of the leather finishing step and the removals of N−NH_3, TN, P-PO_4, COD, BOD and TOC. The authors found no other studies using the microalgae *Tetraselmis* sp. to treat tannery wastewaters. A gap relies on the literature on the influence of light period on the treatment of wastewater from the finishing stage of leather processing using microalgae consortium containing mainly *Tetraselmis* sp..

Therefore, this research attempts to evaluate the growth of a microalgae consortium containing mainly *Tetraselmis* sp. in wastewater generated in the finishing stages (wet-end processing and final finishing). As well as to verify the removal of total nitrogen, total organic carbon, total phosphorus, inorganic carbon, ammonia, BOD and COD comparing different periods of light.

2. Material and methods

2.1. Microalgae selection and growth

Microalgae consortium was collected in a deactivated decanter in a wastewater treatment plant (WWTP) of complete tannery (that processes leather from beamhouse stage to finishing) in the city of Montenegro/RS. This microalgae consortium was chosen due to its adaptation ability in the tannery effluent. The microalgae consortium was grown in medium Tris-Acetate-Phosphate (TAP) containing (mg/L): 2420.0 $H_2NC(CH_2OH)_3$, 375.0 NH_4Cl, 100.0 $MgSO_4 \cdot 7H_2O$, 50.0 $CaCl_2 \cdot 2H_2O$, 288.0 K_2HPO_4, 144.0 KH_2PO_4, 50.0 $Na_2EDTA \cdot 2H_2O$, 22.0 $ZnSO_4$ $7H_2O$, 11.4 H_3BO_3, 5.0 $MnCl_2 \cdot 4H_2O$, 5.0 $FeSO_4 \cdot 7H_2O$, 1.6 $CoCl_2 \cdot 6H_2O$, 1.6 $CuSO_4 \cdot 5H_2O$, 1.1 $(NH_4)6MoO_3$, and 1 mL CH_3COOH (Gorman and Levine, 1965). Microalgae consortium cultivations were carried out in photobioreactors (250 mL Erlenmeyer flask) under continuous aeration at a flow rate of 1 L min^{-1} of compressed air at ambient temperature under continuous light (3910 lux) by white fluorescent lamps. In our earlier study, the authors identified that the species that were present in the largest amount in this microalgae consortium tested was *Tetraselmis* sp. (Pena et al., 2018).

2.2. Effluent selection and compositions

The raw effluent (R) and the secondary effluent (S) from primary physicochemical treatment (coagulation-flocculation-sedimentation) followed by secondary biological treatment (activated sludge-sedimentation) were collected from a tannery that processes leather from wet-blue to finished leather, located in the city of Novo Hamburgo (29°42′58.1"S 51°06′32.7"W), in the State of Rio Grande do Sul, Brazil.

Two different compositions of mixtures were prepared:

50R50S: 2000 mL of the raw effluent, 2000 mL of secondary effluent and 400 mL of the inoculum of microalgae consortium, totaling 4400 mL;

75R25S: 3000 mL of the raw effluent, 1000 mL of secondary effluent and 400 mL of the microalgae consortium inoculum, totaling 4400 mL.

Microalgae consortium in its exponential growth phase (10 days of cultivation with TAP medium) was used as inoculum for the experiments with effluents. The leather industry effluent was not treated before inoculation. The characterization in terms of pH, phosphorus, ammonia, total nitrogen, chemical oxygen demand (COD), and biological oxygen demand (BOD) inorganic carbon and total organic carbon of wastewater generated in the tanneries that process the leather from wet-blue to finished leather was performed before starting each experiment.

2.3. Culture conditions

Microalgae consortium was cultivated in the different compositions mentioned above, in three conditions: (i) constant light (24-light), (ii) 12 h of light and 12 h of darkness (12-light); and, (iii) without light (0-light). Experiments were performed in acrylic photobioreactors with a volume of 5L and 10 cm in diameter. A stone diffuser, connected to an air compressor, was placed inside the photobioreactors to perform air dispersion. The cultivations were carried out under constant aeration with a flow of 1 L min^{-1} of compressed air at ambient temperature. The experiments were performed until some nutrient was removed entirely or until it's 20 days of cultivation. The three conditions were performed in duplicate.

2.4. Monitoring of the microalgae growth and removal of contaminants

To monitoring the growth of the consortium, an initial scan was performed on the spectrophotometer (T80+ UV/vis, PG Instruments). The highest absorbance (Abs) was verified at 570 nm. Therefore, in order to obtain the biomass concentration, first dilutions were performed (TAP culture microalgae consortium) in 10 mL flasks to get absorbance values between 0.1 and 1.0. Then, the optical density at 570 nm of the samples was measured. Finally, the samples were filtered and dried to constant weight in the oven for biomass concentration quantification (g L^{-1}) and correlation with the measured absorbance. A calibration curve (Abs x concentration) was created (Ramirez et al., 2014). During the cultivation period, optical density was analyzed to determine the growth of the microalgae consortium. Samples were collected and filtered using a vacuum pump and glass fiber microfilters (MN GF-3) with 0.6 μm pores. The optical density of tannery wastewater was measured and discounted from the values of the optical density found in the cultivation because it is turbidity that will interfere with the quantification of biomass.

Phosphorus determination was performed according to NBR 12772 (Apha, 2005) (Standard Methods for the Examination of Water and Wastewater, 2005), ammonia (N−NH_3) by ion chromatography (Metrohm Ion Chromatograph Basic IC Plus METROHM, 20000).

Total nitrogen, inorganic carbon (IC), and total organic carbon (TOC) determinations were performed on a Shimadzu TOC-L model carbon analyzer equipped with a total nitrogen determination accessory (TNM-L) and an autosampler (OCT-L). Oxidation of the sample occurs through its catalytic combustion at 680 °C and detection is by non-dispersive infrared (NDIR). Synthetic air 4.7 (White Martins brand) at 150 mL min^{-1} was used as the carrier gas.

Biochemical oxygen demand (BOD_5) measurements were performed with a VELP Scientifica pressure sensor (BOD Sensor) equipped with a stirring system (System 6). After mounting, the vials were incubated at 20 °C for 5 days. Chemical oxygen demand (COD) was performed by the closed reflux colorimetric method (Apha, 2005) where the organic and inorganic materials present in the sample are oxidized by potassium dichromate oxidizing agent ($K_2Cr_2O_7$). COD is quantified by being linearly proportional to the color change of the medium as chromium is reduced (Cr^{6+} to Cr^{3+}).

These measurements were carried out at the beginning and at the end of the experiments. The results are presented as mean ± standard deviation. Statistical analysis was performed using Microsoft Excel (2012 version). Statistical significance values for the means were evaluated using one-way ANOVA (test-F of analysis of variance). Differences were accepted as significant when p-value was <0.05.

3. Results and discussion

3.1. Growth of microalgae consortium in tannery effluents by different photoperiods

Biomass growth curves over the 19 days of 24-light, 12-light, and 0-light cultivation for 50R50S and 75R25S compositions are shown in Fig. 1. In Fig. 1A, the lag phase has been observed on the 1st and up to the 3rd day for 75R25S and 50R50S, respectively. Then the exponential growth phase was observed until the 11th and 7th (75R25S and 50R50S, respectively).The maximum biomass concentration of the microalgae consortium found in the 24-light culture was 1.40 g L^{-1} on day 11th, for 75R25S. For 50R50S, the maximum concentration was 1.04 g L^{-1} on the 7th day. After reaching the maximum concentration, the microalgae culture entered the decline phase, which indicates that there has already been a reduction in the concentration of nutrients in the medium and that more cells are dying than reproducing. The most significant growth in 24-light culture was due to the longer time of light incision in the culture when compared to 12-light and 0-light culture. Higher biomass growth was also obtained by Bazdar et al. (2018) when cultivating the microalgae *Chlorella vulgaris* in municipal wastewater under continuous light compared to two light/dark regimes (12h/12h and 16h/8h).

Both values (1.40 g L^{-1} and 1.4 g L^{-1}) for maximum concentration of the microalgae consortium are higher than those found by Fontoura et al., 02017 with beamhouse wastewater, where for *Scenedesmus* sp. the maximal biomass concentration achieved was 0.90 g L^{-1} in light intensity of 182.5 μmol photons m^{-2} s^{-1} (about 13500 lux, more than three times the light intensity used in this study). The higher growth of the microalgae consortium in tannery wastewater compared to the microalgae *Scenedesmus* sp. can be explained by the previous adaptation of the microalgae consortium in this type of effluent, as it was collected in a deactivated decanter in a wastewater treatment unit of a complete tannery. Additionally, the growth of the microalgae consortium was higher for 75R25S medium. It was due to the higher proportion of raw effluent, meaning that the initial nutrient concentrations are higher than in 50R50S.

From Fig. 1B, 2-light cultivation, it is possible to observe that the 50R50S effluent allowed more significant growth of microalgae (maximal biomass concentration of 0.30 g L^{-1}) when compared to 75R25S (maximal biomass concentration of 0.14 g L^{-1}). The 75R25S is a darker mixture due to its higher concentration of raw wastewater. As the light was reduced to 12 h, the difficulty of performing photosynthesis increased. Therefore, the higher concentration of the effluent did not favor growth because it was counterbalanced by the high turbidity hindering the penetration of light in the medium. It is possible to see in this figure the oscillatory growth of culture, indicating that the microalgae consortium is trying to adapt to the tannery wastewater.

Among the three cultures, 0-light (Fig. 1C) presented the worst performance, with lowest biomass concentration values in both compositions: 0.038 g L^{-1} for 50R50S and 0.033 g L^{-1} for 75R25S. Thus, as expected, it was verified that the reduction of the light exposure time had a negative effect on the growth of the microalgae consortium. This condition presented high values of standard deviation due to the low growth values presented.

Gladue and Maxey (1994) studied 121 strains of microalgae containing several species and only 57 grew in heterotrophic culture, of which 44 were *Tetraselmis*, 6 *Chlorella*, 4 *Nannochloropsis* and 3 *Dunaliella*. Most species grew in the presence of glucose, and few algae grew under heterotrophic conditions with glycerol or organic acids. Azma et al. (2011) obtained high performance on the growth of the *Tetraselmis suecica* microalgae in heterotrophic cultivation in an optimized culture medium (28.88 g L^{-1} of cell at 6.19 g L^{-1} glucose, 9.19 g L^{-1} peptone, 5.99 g L^{-1} yeast extract, and 3.01 g L^{-1} meat extract). Supposedly, the tannery wastewaters used in our work do not have all the necessary nutrients and in adequate quantities for the growth of the consortium of microalgae without light or does not have has strains that can develop without light.

Fig. 2 shows a microscopic image of the microalgae consortium and the predominant microalgae (Tetraselmis sp.) before being placed in tannery wastewater.

3.2. Nutrient removal

The initial and final concentrations (average of duplicates ± standard deviations) of P-PO_4, TN, N−NH_3, COD, TOC, IC, and BOD are shown in Table 2 for 24-light and 12-light. The parameters analyzed for the 50R50S and 75R25S compositions, under both conditions (24-light and 12-light cultures), showed a significant reduction (p-value<0,05) during the cultivation.

Complete removal of the ammonia in 24-light could have been favored by the increase of the pH during the culture. The initial pH in the composition 50R50S was 7.98, and changed to 8.66 on the final day. Likewise, to 75R25S, it changed from 8.07 to 9.06. The assimilation of nitrogen by microalgae is in the form of nitrates, nitrites and mainly ammonia. The increase of the pH during the microalgae photosynthesis metabolism favors the more significant presence of NH_3 (free ammonia), because at pH close to 9.25 approximately half of the ammonia is in the form of free ammonia, that quickly volatilizes, and the other half in the form of NH_4^+ (Markou and Georgakakis, 2011). In 12-light culture, the pH also increased for both for the composition 50R50S and 75R25S, from 7.60 for 8.47 and 7.81 for 8.62, respectively.

The decrease of TOC on 24-light and 12-light indicates that the mixotrophic culture was established because there was the consumption of organic carbon associated with the inorganic carbon fixation (Patel et al., 2020). The organic carbon was reduced from 86.48 mg L^{-1} to 46.21 mg L^{-1} for 50R50S and 96.81 mg L^{-1} to 63.30 mg L^{-1} for 75R25S in the 24-light. In the 12-light experiment, the inorganic carbon reduction was from 80.71 mg L^{-1} to 41.83 mg L^{-1} under the condition of 50R50S and 87.84 mg L^{-1} for 57.26 mg L^{-1} under the condition of 75R25S.

In 12-light cultivation, there was a more significant reduction of organic carbon for both 50R50S and 75R25S compositions, indicating that the less light present in cultivation, the higher the consumption of organic carbon, because of the lack of light disadvantages photosynthesis. The same was observed for the increase of wastewater concentration as the more concentrated, the darker the wastewater, and the higher the reduction of organic carbon. *Tetraselmis* microalgae growth rates were slightly higher under mixotrophic conditions compared to heterotrophic culture systems (Day and Tsavalos, 1996).

It explains the higher growth in 24-light and 12-light when compared to 0-light cultures, as mixotrophic cultivation achieves higher biomass production in a short period than other growth modes due to the simultaneous execution of the pentose phosphate and photosynthesis pathways (Patel et al., 2019). The most substantial growth on 24-light experiments compared to 0-light shows that these microalgae require light for organic carbon assimilation

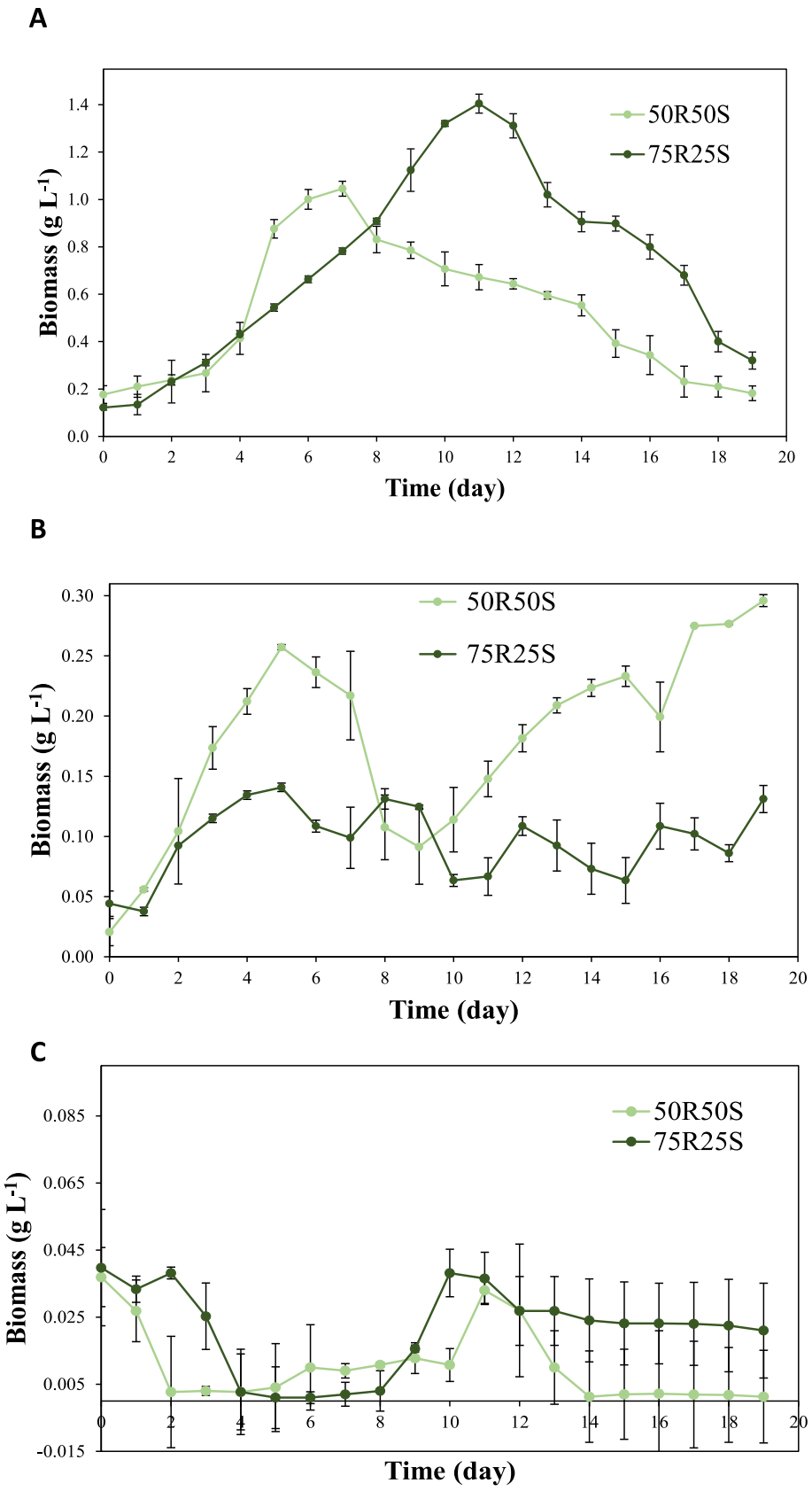

Fig. 1. Average growth of the microalgae consortium in 24-light (A), 12-light (B) and 0-light (C) culture in tannery effluents in compositions 50R50S and 75R25S.

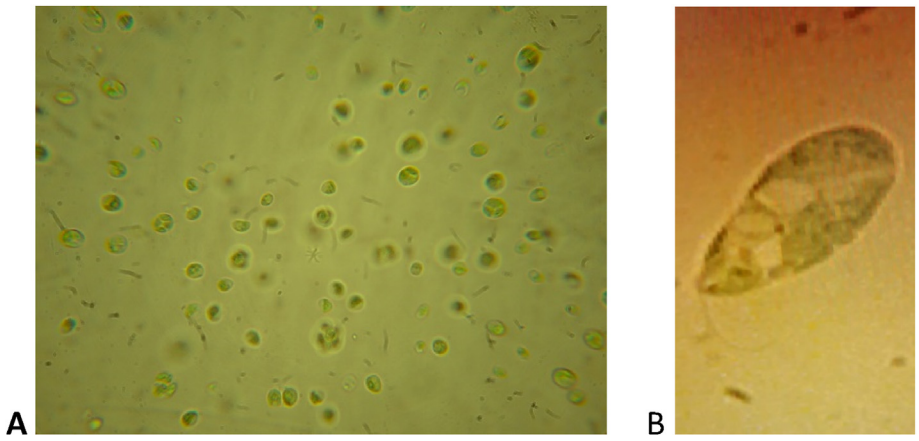

Fig. 2. Optical microscopy of the (A) microalgae consortium (10x) and (B) predominant microalgae (40x).

due to their poor growth in the dark, under organic carbon conditions.

Nitrogen and phosphorus are the other two main elements for the growth of the microalgae. Its composition in the TAP medium was 98.0 mg L^{-1} for nitrogen and 15.98 mg L^{-1} for phosphorus, with a N:P ratio 6.13. It is observed that the P-PO4 was about 100% depleted after the 19 days of cultivation, and this may have resulted in the incomplete removal of nitrogen and other compounds in the wastewater. For the microbiological processes to work effectively, it needs to provide a right balance of essential nutrients to the microorganisms. The N:P initial ratio was above 57.0 for the 50R50S compositions and above 33.0 for the 75R25Scompositions. Effluent nitrogen composition values are well above those found in TAP culture medium.

The 24-light culture showed significant differences between the removals of the 50R50S and 75R25S compositions for all parameters. The 75R25S composition presented higher removal values for COD, TOC, and BOD. The 12- light culture showed differences between the removals of the 50R50S and 75R25S compositions for phosphorus P-PO_4 and COD. There was more significant removal of P-PO_4 (45.17%) in the 50R50S composition and higher COD removal (37.50%) in the 75R25S composition.

The comparison of parameter removal in relation to the photoperiods can also be observed in Table 2.The removals were statistically different (p-value < 0.05) for P-PO_4, N–NH_3, COD and BOD_5, between the 24-light and 12-light cultures in the 50R50S composition, while TN and TOC had significantly the same removals. Removals of P-PO_4, N–NH_3, and COD were more substantial in the 24-light culture.

In the 75R25S composition, the P-PO_4, TN, N–NH_3, and COD showed a significant statistical difference for the 24-light (higher removal) and 12-light (lower removal) cultures. This may have occurred due to the ease of the microalgae consortium to perform photosynthesis instead of consuming organic carbon, facilitating the autotrophic growth in the 24-light cultivation, as it presents higher light availability, promoting the consortium growth and, therefore, more significant removal of the available nutrients.

Most species of microalgae are obligatory autotrophic, not growing in the absence of light and carbon dioxide (Perez-Garcia and Bashan, 2015). In this way, the cultivation with greater availability of light (24-light) presented better results, affirming the results obtained in the growth of the cultures that were also bigger in the composition 24-light.

In the 0-light culture, there was no significant reduction of the parameters analyzed, for both effluents compositions (Table 3), except for 75R25S, where ammonia, IC, and phosphorus were

Table 2
Average of the initial and final concentrations of the parameters analyzed in the 24-light and 12-light culture for the compositions 50R50S and 75R25S.

	Initial	Final	Reduction (%)	Initial	Final	Reduction (%)
		24-light			12-light	
	50R50S					
pH	7.98	8.66	–	7.60	8.47	–
P-PO_4 (ppm)	1.83 ± 0.05^{a}	0.04 ± 0.01^{b}	$97.64^{A,*}$	1.74 ± 0.01^{1}	0.95 ± 0.01^{2}	$45.17^{A,\#}$
TN (ppm)	103.80 ± 0.31^{a}	29.54 ± 3.84^{b}	$71.74^{A,*}$	104.55 ± 4.69^{1}	48.84 ± 0.65^{2}	$53.28^{A,*}$
N–NH_3 (ppm)	69.66 ± 2.45^{a}	0.00 ± 0.00^{b}	$100.00^{A,*}$	71.25 ± 0.40^{1}	21.26 ± 2.14^{2}	$70.16^{A,\#}$
COD (ppm)	814.00 ± 28.28^{a}	404.00 ± 28.28^{b}	$50.37^{A,*}$	685.00 ± 7.07^{1}	455.00 ± 7.07^{2}	$33.58^{A,\#}$
TOC (ppm)	87.18 ± 2.95^{a}	69.72 ± 1.82^{b}	$20.02^{A,*}$	93.79 ± 4.57^{1}	65.07 ± 4.95^{2}	$30.62^{A,*}$
IC (ppm)	86.48 ± 4.29^{a}	46.22 ± 1.97^{b}	$46.65^{A,*}$	80.71 ± 3.98^{1}	41.83 ± 1.58^{2}	$48.17^{A,*}$
BOD_5 (ppm)	1130.00 ± 28.28^{a}	940.00 ± 14.14^{b}	$16.81^{A,*}$	1090.00 ± 28.28^{1}	820.00 ± 14.14^{2}	$24.77^{A,*}$
	75R25S					
pH	8.07	9.06	–	7.81	8.62	–
P-PO_4 (ppm)	3.41 ± 0.05^{a}	0.15 ± 0.004^{b}	$95.54^{B,*}$	2.66 ± 0.06^{1}	2.01 ± 0.03^{2}	$24.22^{B,\#}$
TN (ppm)	148.22 ± 2.04^{a}	61.01 ± 0.34^{b}	$58.84^{B,*}$	117.36 ± 5.63^{1}	68.45 ± 1.22^{2}	$41.68^{A,\#}$
N–NH_3 (ppm)	82.74 ± 0.37^{a}	0.00 ± 0.00^{b}	$100.00^{B,*}$	77.21 ± 5.02^{1}	33.69 ± 3.22^{2}	$56.37^{A,\#}$
COD (ppm)	1120.00 ± 0.00^{a}	485.00 ± 7.07^{b}	$56.70^{B,*}$	1120.00 ± 0.00^{1}	700.00 ± 14.14^{2}	$37.50^{B,\#}$
TOC (ppm)	103.59 ± 4.96^{a}	72.27 ± 4.24^{b}	$31.35^{B,*}$	100.89 ± 1.31^{1}	67.45 ± 3.96^{2}	$33.14^{A,*}$
IC (ppm)	96.81 ± 2.33^{a}	63.30 ± 4.96^{b}	$34.61^{A,*}$	87.84 ± 1.78^{1}	57.26 ± 5.62^{2}	$34.81^{A,*}$
BOD_5 (ppm)	1330.00 ± 28.28^{a}	1055.00 ± 7.0^{b}	$20.68^{B,*}$	1270.00 ± 56.57^{1}	940.00 ± 14.14^{2}	$25.98^{A,*}$

Lowercase, numbers, * and # indicate statistical differences between lines (50R50S and 75R25S) ($p < 0.05$).
Uppercase indicate statistical differences between columns (24-light and 12-light) ($p < 0.05$).

Table 3
Average of the initial and final concentrations of the parameters analyzed in the 0-light culture for the compositions 50R50S and 75R25S.

50R50S			
	Initial	Final	Reduction (%)
pH	7.68	8.31	–
P-PO_4 (ppm)	1.49 ± 0.10[a]	1.08 ± 0.06[a]	27.71[A]
TN (ppm)	103.80 ± 0.31[a]	93.58 ± 3.43[a]	9.85[A]
N–NH_3 (ppm)	74.44 ± 0.45[a]	72.66 ± 0.59[a]	2.39[A]
COD (ppm)	820.00 ± 14.14[a]	780.00 ± 0.00[a]	4.88[A]
TOC (ppm)	90.44 ± 1.41[a]	84.66 ± 3.54[a]	6.39[A]
IC (ppm)	77.61 ± 0.41[a]	68.31 ± 1.25[b]	11.99[A]
BOD_5 (ppm)	1150.00 ± 45.43[a]	1080.00 ± 56.57[a]	6.09[A]
75R25S			
	Initial	Final	Reduction (%)
pH	7.96	8.63	–
P-PO_4 (ppm)	3.66 ± 0.16[a]	2.58 ± 0.02[b]	29.55[B]
TN (ppm)	118.01 ± 2.68[a]	104.14 ± 3.90[a]	11.75[A]
N–NH_3 (ppm)	76.37 ± 0.47[a]	73.08 ± 0.40[b]	4.31[B]
COD (ppm)	1110.00 ± 14.14[a]	1060.00 ± 42.43[a]	4.50[A]
TOC (ppm)	112.15 ± 0.87[a]	106.45 ± 5.52[a]	5.08[A]
IC (ppm)	83.01 ± 0.66[a]	70.23 ± 0.35[b]	15.40[B]
BOD_5 (ppm)	1350.00 ± 14.14[a]	1320.00 ± 28.28[a]	2.22[A]

Lowercase indicate statistical differences between lines ($p < 0.05$).
Uppercase indicate statistical differences between columns ($p < 0.05$).

significantly removed. The ammonia removal again could have been facilitated by the basic pH in this composition. This proves that the microalgae consortium was unable to establish itself in the tanning effluent without the presence of light.

4. Conclusions

This work carried out the cultivation of a microalgae consortium in tannery wastewater from the finishing stage in two compositions (75R25S and 50R50S). The removal of total nitrogen, total organic carbon, total phosphorus, inorganic carbon, ammonia, BOD, and COD were evaluated and cultivations in different light periods were compared (24-light, 12-light and 0-light). Maximum growth was achieved in the 24-light wastewater in the composition 75R25S (1.40 g L^{-1}). The 24-light culture exhibits higher removals of N–NH_3 (100%), COD (56.70%), TOC (31.35%) and BOD_5 (20.68%) in the 75R25S composition when compared to the 50R25S composition. Also, it was demonstrated that 24-light cultivations favored the removal of P-PO_4 (95.54% and 97.64%), TN (58.84% and 71.74%), N–NH_3 (both 100%), and COD (56.70% and 56.70%), when compared to the 12-light culture for 75R25S and 50R50S, respectively. In this way, it is more appropriate to use the 75R25S composition in 24-light for the treatment of the effluent, since it has a higher wastewater load of nutrients for the microalgae consortium. It has not been able to develop in cultivation without light, maybe due to the lack of carbon in the quantities and the proper form so that they can be converted into energy in order to supply the lack of light of the heterotrophic medium. Therefore, the cultivation of the microalgae consortium in wastewater without pretreatment has shown to be a promising path for the wastewater treatment of the leather finishing step to remove nutrients and generate biomass. The biomass generated can be exploited for different applications, such as biodiesel, biofuel, biogas and bio-hydrogen.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Aline C.C. Pena: Conceptualization, Methodology, Resources, Writing - original draft. **Caroline B. Agustini:** Formal analysis, Software, Writing - review & editing. **Luciane F. Trierweiler:** Validation, Supervision, Writing - review & editing. **Mariliz Gutterres:** Validation, Supervision, Writing - review & editing, Project administration, Funding acquisition.

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References

Ajayan, K.V., Selvaraju, M., Unnikannan, P., Sruthi, P., 2015. Phycoremediation of tannery wastewater using microalgae Scenedesmus species. Int. J. Phytoremediation 17, 907–916. https://doi.org/10.1080/15226514.2014.989313.

Aketo, T., Hoshikawa, Y., Nojima, D., Yabu, Y., Maeda, Y., Yoshino, T., Takano, H., Tanaka, T., 2020. Selection and characterization of microalgae with potential for nutrient removal from municipal wastewater and simultaneous lipid production. J. Biosci. Bioeng. https://doi.org/10.1016/J.JBIOSC.2019.12.004.

Apha, 2005. Standard Methods Forthe Examination of Water and Wastewater, twentieth ed. (Washington DC).

Aquim, P.M., Hansen, É., Gutterres, M., 2019. Water reuse: an alternative to minimize the environmental impact on the leather industry. J. Environ. Manag. 230, 456–463. https://doi.org/10.1016/j.jenvman.2018.09.077.

Azma, M., Mohamed, M.S., Mohamad, R., Rahim, R.A., Ariff, A.B., 2011. Improvement of medium composition for heterotrophic cultivation of green microalgae, Tetraselmis suecica, using response surface methodology. Biochem. Eng. J. 53, 187–195. https://doi.org/10.1016/J.BEJ.2010.10.010.

Ballen-Segura, M., Hernandez Rodriguez, L., Parra Ospina, D., Vega Bolaños, A., Perez, K., 2016. Using Scenedesmus sp. for the phycoremediation of tannery wastewater. Tecciencia 11, 69–75. https://doi.org/10.18180/tecciencia.2016.21.11.

Bazdar, E., Roshandel, R., Yaghmaei, S., Mardanpour, M.M., 2018. The effect of different light intensities and light/dark regimes on the performance of photosynthetic microalgae microbial fuel cell. Bioresour. Technol. 261, 350–360. https://doi.org/10.1016/J.BIORTECH.2018.04.026.

Benvenuti, J., Capeletti, L.B., Gutterres, M., dos Santos, J.H.Z., 2018. Hybrid sol–gel silica adsorbent materials synthesized by molecular imprinting for tannin removal. J. Sol. Gel Sci. Technol. 85, 446–457. https://doi.org/10.1007/s10971-017-4564-z.

Das, C., Naseera, K., Ram, A., Meena, R.M., Ramaiah, N., 2017. Bioremediation of

tannery wastewater by a salt-tolerant strain of Chlorella vulgaris. J. Appl. Phycol. 29, 235–243. https://doi.org/10.1007/s10811-016-0910-8.
Das, C., Ramaiah, N., Pereira, E., Naseera, K., 2018. Efficient bioremediation of tannery wastewater by monostrains and consortium of marine *Chlorella* sp. and *Phormidium* sp. Int. J. Phytoremediation 20, 284–292. https://doi.org/10.1080/15226514.2017.1374338.
Day, J.G., Tsavalos, A.J., 1996. An investigation of the heterotrophic culture of the green alga Tetraselmis. J. Appl. Phycol. 8, 73–77. https://doi.org/10.1007/BF02186225.
Duan, B., Wang, Q., Wang, X., Li, Y., Zhang, M., Diao, S., 2019. Flame retardance of leather with flame retardant added in retanning process. Results Phys 15, 102717. https://doi.org/10.1016/J.RINP.2019.102717.
Dunn, K., Maart, B., Rose, P., 2013. Arthrospira (Spirulina) in tannery wastewaters. Part 2: evaluation of tannery wastewater as production media for the mass culture of Arthrospira biomass. WaterSA 39, 279–284. https://doi.org/10.4314/wsa.v39i2.12.
Fontoura, J.T. da, Rolim, G.S., Farenzena, M., Gutterres, M., 2017. Influence of light intensity and tannery wastewater concentration on biomass production and nutrient removal by microalgae Scenedesmus sp. Process Saf. Environ. Protect. 1, 355–362. https://doi.org/10.1016/j.psep.2017.07.024.
Fuck, W.F., Lopes, F.C., Brandelli, A., Gutterres, M., 2018. Screening of natural dyes from filamentous fungi and leather dyeing with monascus purpureus extract. J. Soc. Leather Technol. Chem. 102, 69–74.
Gladue, R.M., Maxey, J.E., 1994. Microalgal feeds for aquaculture. J. Appl. Phycol. 6, 131–141. https://doi.org/10.1007/BF02186067.
Gomes, C.S., Piccin, J.S., Gutterres, M., 2016. Optimizing adsorption parameters in tannery-dye-containing effluent treatment with leather shaving waste. Process Saf. Environ. Protect. 99, 98–106. https://doi.org/10.1016/j.psep.2015.10.013.
Gorman, D.S., Levine, R.P., 1965. Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of chlamydomonas reinhardi. Biochemistry 54, 1665–1669. https://doi.org/10.1073/pnas.54.6.1665.
Gutterres, M., Ortiz-Monsalve, S., Fontoura, J., Benvenuti, J., 2015. Characterization of raw wastewater from tanneries. J. Soc. Leather Technol. Chem. 99, 280–287.
Han, Y., Hu, J., Xin, Z., 2019. Facile preparation of high solid content waterborne polyurethane and its application in leather surface finishing. Prog. Org. Coating 130, 8–16. https://doi.org/10.1016/J.PORGCOAT.2019.01.031.
Khalid, A.A.H., Yaakob, Z., Abdullah, S.R.S., Takriff, M.S., 2019. Assessing the feasibility of microalgae cultivation in agricultural wastewater: the nutrient characteristics. Environ. Technol. Innov. 15, 100402 https://doi.org/10.1016/J.ETI.2019.100402.
Kuppusamy, S., Jayaraman, N., Jagannathan, M., Kadarkarai, M., Aruliah, R., 2017. Electrochemical decolorization and biodegradation of tannery effluent for reduction of chemical oxygen demand and hexavalent chromium. J. Water Process Eng. 20, 22–28. https://doi.org/10.1016/J.JWPE.2017.09.008.
Lai, S., Jin, Y., Li, H., Zhang, L., Yin, X., 2017. Application of Y-shaped polyurethane and polyacrylic acid as a complex retanning agent in aldehyde-tanned goat leather. JALCA (J. Am. Leather Chem. Assoc.) 112.
Li, X., Yang, C., Zeng, G., Wu, S., Lin, Y., Zhou, Q., Lou, W., Du, C., Nie, L., Zhong, Y., 2020. Nutrient removal from swine wastewater with growing microalgae at various zinc concentrations. Algal Res 46, 101804. https://doi.org/10.1016/J.ALGAL.2020.101804.
Markou, G., Georgakakis, D., 2011. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review. Appl. Energy 88, 3389–3401. https://doi.org/10.1016/J.APENERGY.2010.12.042.
Mella, B., Benvenuti, J., Oliveira, R.F., Gutterres, M., 2019. Preparation and characterization of activated carbon produced from tannery solid waste applied for tannery wastewater treatment. Environ. Sci. Pollut. Res. 26, 6811–6817. https://doi.org/10.1007/s11356-019-04161-x.
Nayak, J.K., Ghosh, U.K., 2019. Post treatment of microalgae treated pharmaceutical wastewater in photosynthetic microbial fuel cell (PMFC) and biodiesel production. Biomass Bioenergy 131, 105415. https://doi.org/10.1016/J.BIOMBIOE.2019.105415.
Onem, E., 2018. Quality properties of leather produced in water and supercritical fluid (SCF) media. J. CO2 Util. 23, 75–79. https://doi.org/10.1016/J.JCOU.2017.10.019.
Ortiz-Monsalve, S., Valente, P., Poll, E., Jaramillo-García, V., Pegas Henriques, J.A., Gutterres, M., 2019. Biodecolourization and biodetoxification of dye-containing wastewaters from leather dyeing by the native fungal strain Trametes villosa SCS-10. Biochem. Eng. J. 141, 19–28. https://doi.org/10.1016/J.BEJ.2018.10.002.
Patel, A.K., Joun, J.M., Hong, M.E., Sim, S.J., 2019. Effect of light conditions on mixotrophic cultivation of green microalgae. Bioresour. Technol. 282, 245–253. https://doi.org/10.1016/J.BIORTECH.2019.03.024.
Patel, A.K., Choi, Y.Y., Sim, S.J., 2020. Emerging prospects of mixotrophic microalgae: way forward to sustainable bioprocess for environmental remediation and cost-effective. biofuels. Technol. 300 https://doi.org/10.1016/j.biortech.2020.122741.
Pena, A. de C.C., Bertoldi, C.F., Fontoura, J.T. da, Trierweiler, L.F., Gutterres, M., 2019. Consortium of microalgae for tannery effluent treatment. Braz. Arch. Biol. Technol. 62 https://doi.org/10.1590/1678-4324-2019170518.
Pena, A.D.C.C., Schaumlöffel, L.D.E.S., Trierweiler, L.F., Gutterres, M., 2018. Tetraselmis sp . Isolated from a microalgae consortium for tannery wastewater treatment. J. Soc. Leather Technol. Chem. 102, 10–13.
Perez-Garcia, O., Bashan, Y., 2015. Microalgal heterotrophic and mixotrophic culturing for bio-refining: from metabolic routes to techno-economics. In: Algal Biorefineries. Springer International Publishing, Cham, pp. 61–131. https://doi.org/10.1007/978-3-319-20200-6_3.
Puchana-Rosero, M.J., Lima, E.C., Mella, B., Da Costa, D., Poll, E., Gutterres, M., 2018. A coagulation-flocculation process combined with adsorption using activated carbon obtained from sludge for dye removal from tannery wastewater. J. Chil. Chem. Soc. 63, 3867–3874. https://doi.org/10.4067/s0717-97072018000103867.
Ramirez, N.N.V., Farenzena, M., Trierweiler, J.O., 2014. Growth of microalgae Scenedesmus sp in ethanol vinasse. Braz. Arch. Biol. Technol. 57, 630–635. https://doi.org/10.1590/S1516-8913201401791.
Ran, W., Wang, H., Liu, Y., Qi, M., Xiang, Q., Yao, C., Zhang, Y., Lan, X., 2019. Storage of starch and lipids in microalgae: biosynthesis and manipulation by nutrients. Bioresour. Technol. 291, 121894 https://doi.org/10.1016/J.BIORTECH.2019.121894.
Romero-Dondiz, E.M., Almazán, J.E., Rajal, V.B., Castro-Vidaurre, E.F., 2016. Comparison of the performance of ultrafiltration and nanofiltration membranes for recovery and recycle of tannins in the leather industry. J. Clean. Prod. 135, 71–79. https://doi.org/10.1016/J.JCLEPRO.2016.06.096.
Saranya, D., Shanthakumar, S., 2020. An integrated approach for tannery effluent treatment with ozonation and phycoremediation: a feasibility study. Environ. Res. 183, 109163 https://doi.org/10.1016/J.ENVRES.2020.109163.
Saranya, D., Shanthakumar, S., 2019. Green microalgae for combined sewage and tannery effluent treatment: performance and lipid accumulation potential. J. Environ. Manag. 241, 167–178. https://doi.org/10.1016/J.JENVMAN.2019.04.031.
Sathish, M., Subramanian, B., Rao, J.R., Fathima, N.N., 2019. Deciphering the role of individual retanning agents on physical properties of leathers. J. Am. Leather Chem. Assoc. 114, 94–102.
Sawalha, H., Alsharabaty, R., Sarsour, S., Al-Jabari, M., 2019. Wastewater from leather tanning and processing in Palestine: characterization and management aspects. J. Environ. Manag. 251, 109596 https://doi.org/10.1016/J.JENVMAN.2019.109596.
Song, C., Han, X., Qiu, Y., Liu, Z., Li, S., Kitamura, Y., 2020. Microalgae carbon fixation integrated with organic matters recycling from soybean wastewater: effect of pH on the performance of hybrid system. Chemosphere 248, 126094. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126094.
Standard Methods for the Examination of Water and Wastewater, 2005.
Sun, X., Jin, Y., Lai, S., Pan, J., Du, W., Shi, L., 2018. Desirable retanning system for aldehyde-tanned leather to reduce the formaldehyde content and improve the physical-mechanical properties. J. Clean. Prod. 175, 199–206. https://doi.org/10.1016/J.JCLEPRO.2017.12.058.
Sundaramoorthy, B., Thiagarajan, K., Mohan, Shalini, Mohan, Sankari, Rajendra Rao, P., Ramamoorthy, S., Chandrasekaran, R., 2016. Biomass characterisation and phylogenetic analysis of microalgae isolated from estuaries: role in phycoremediation of tannery effluent. Algal Res 14, 92–99. https://doi.org/10.1016/j.algal.2015.12.016.
Tamersit, S., Bouhidel, K.-E., Zidani, Z., 2018. Investigation of electrodialysis antifouling configuration for desalting and treating tannery unhairing wastewater: feasibility of by-products recovery and water recycling. J. Environ. Manag. 207, 334–340. https://doi.org/10.1016/j.jenvman.2017.11.058.
Tamilselvi, A., Jayakumar, G.C., Sri Charan, K., Sahu, B., Deepa, P.R., Kanth, S.V., Kanagaraj, J., 2019. Extraction of cellulose from renewable resources and its application in leather finishing. J. Clean. Prod. 230, 694–699. https://doi.org/10.1016/J.JCLEPRO.2019.04.401.
Urrutia, C., Yañez-Mansilla, E., Jeison, D., 2019. Bioremoval of heavy metals from metal mine tailings water using microalgae biomass. Algal Res 43, 101659. https://doi.org/10.1016/J.ALGAL.2019.101659.
Winter, C., Agustini, C.B., RS, M.E., Gutterres, M., 2018. Behavior of polymer films and its blends for leather finishing. Trends Text. Eng. Fash. Technol. 1, 1–10. https://doi.org/10.31031/tteft.2018.01.000518.
Zanoni, M.V.B., Yanamaka, H., 2016. Corantes: Caracterização química, toxicológica, métodos de detecção e tratamento.